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Original Article

Relationship between *CTLA-4* +49A>G polymorphism and the risk of lung adenocarcinoma in a Chinese Han population

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Abstract: Background: Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a transmembrane protein on the activated T-cell surface. It can inhibit T-cell proliferation and activation and it increases tumor susceptibility. Some reports have associated *CTLA-4* +49A>G polymorphism with certain cancers, but the associations with lung cancer have been inconsistent. Therefore, we aimed to investigate the associations between *CTLA-4* +49A>G polymorphism and the risk of lung cancer, as well as the risks of different pathological types of lung cancer. Methods: A total of 549 lung cancer patients and 611 healthy controls were included in this study. Taqman real-time polymerase chain reaction was used to analyze *CTLA-4* polymorphisms. The chi-squared test was used to examine the differences between lung cancer patients and controls. The odds ratio (OR) and its 95% confidence interval (95% CI) were obtained by multivariate conditional logistic regression. Results: When the AA genotype was used as the reference group, the GG genotype was associated with an increased risk of lung cancer (OR = 1.697, 95% CI = 1.095-2.630, P = 0.018). Moreover, in the analysis of *CTLA-4* +49A>G polymorphism and the risks of different pathological types of lung cancer, the GG genotype was associated with an increased risk of lung adenocarcinoma (OR = 2.443, 95% CI = 1.337-4.465; P = 0.004). Under the dominant model of inheritance, the AG+GG genotype was significantly associated with an increased risk of lung adenocarcinoma (OR = 2.095, 95% CI = 1.173-3.741; P = 0.012). Conclusion: The *CTLA-4* +49A>G gene polymorphism is associated with a risk of lung adenocarcinoma in a Chinese Han population.

Keywords: Lung cancer, CTLA-4, gene polymorphism, risk

Introduction

Lung cancer is the most common cancer worldwide and it is the leading cause of cancer-related death among both women and men [1]. Recently, in some western countries, the incidence and mortality rate of lung cancer have stabilized or decreased [2, 3]. However, during the same time, the incidence and mortality rate have consistently and rapidly increased in China [4]. In 2015, lung cancer remained the most common cancer and the leading cause of cancer death in China [5]. Lung cancer is recognized as a multifactorial disease that results from complex interactions between genetics and environmental factors. Some studies suggest that genetics is the main factor in the development of lung cancer [6].

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), also known as CD152, is a transmembrane protein on the activated T-cell surface that is coded by the *CTLA-4* gene [7]. CTLA-4 has a high degree of homology with the co-stimulatory molecule receptor (CD28) on the T-cell surface. They are both members of an immunoglobulin superfamily and competitively bind with the same ligands (CD80 [B7-1] and CD86 [B7-2]). In contrast to the function of CD28, the CTLA-4 molecule binds with the B7 molecule and produces an inhibitory signal, which can block T-cell proliferation and activation in the early stages of tumor formation. Therefore, tumor cells can escape from the attack of the T-cells and, therefore, tumor susceptibility increases [8-10].

Table 1. Characteristics of patients and controls

Characteristic	Subgroup	Cases (n = 549)	Controls (n = 611)	*P*-value
		Number (%)	Number (%)	
Sex	Male	391 (71.22)	433 (70.90)	0.897
	Female	158 (28.78)	178 (29.10)	
Mean age (years ± SD)		60.02±9.937	60.00±10.145	0.970
Smoking status	Ever	385 (70.10)	191 (31.30)	<0.001
	Never	164 (29.90)	420 (68.70)	
Pathological type of cancer	Adenocarcinoma	238 (43.35)		
	Squamous cell	213 (38.80)		
	carcinoma SCLC	98 (17.85)		

Note: SCLC = small cell lung cancer; SD = standard deviation.

Gene functions can be affected by a variety of mutations, including single nucleotide polymorphisms, that result in the development of different types of disease [11]. The *CTLA-4* gene is located on chromosome 2q33. Of all possible polymorphisms for *CTLA-4*, only the +49A>G polymorphism can cause changes in the amino acid sequence of CTLA-4. Therefore, much research has focused on this polymorphism [12]. Previous reports showed that the *CTLA-4* +49A>G polymorphism was associated with several types of cancer, including lung cancer, breast cancer, colorectal cancer, and melanoma. However, results about associations with lung cancer have been inconsistent [7, 11, 13-15]. Therefore, we aimed to investigate the association between *CTLA-4* +49A>G polymorphism and the risk of lung cancer, as well as the risks of different pathological types of lung cancer.

Material and methods

Study participants

All participants were from the Han population in Liaoning Province. A total of 549 lung cancer patients were selected between May 2009 and July 2015 from the First and Fourth Affiliated Hospital of China Medical University for inclusion in this study. All patients had histologically confirmed disease and all patients met the diagnostic criteria for lung cancer established by the World Health Organization. They were diagnosed with primary lung cancer without previous chemotherapy or radiotherapy prior to the beginning of the study. During the same time period, 611 healthy controls without family histories of cancer were randomly selected from the health examination center in the Fourth Affiliated Hospital of China Medical University and The General Hospital of Shenyang Military Region. Face-to-face interviews of the healthy controls and patients were conducted by 2 trained interviewers who collected and recorded pertinent information (e.g., name, age, sex, smoking status, etc.).

DNA extraction and genotyping

First, 5 ml peripheral blood was obtained from each participant; DNA was extracted from the blood using the phenol-chloroform method. (EDTA was used for anticoagulation and the samples were stored at -80°C until further use). Aliquot DNA was diluted to a final concentration of 30 to 100 ng/μl and stored at -20°C until analysis. Taqman real-time polymerase chain reaction (PCR) was used to analyze the *CTLA-4* polymorphism. The PCR protocol included 47 cycles, and the total volume of the reaction mixture was 5 μl: 1 μl of genomic DNA, 2.5 μl of 2× Taqman Master Mix, 0.25 μl of 20× primer and probe mixture, and 1.25 μl of distilled water. The primers were designed by AB applied biosystems TaqMan SNP Genotyping Assays (Assay ID: C_2415786_20, RS Number: rs231775, Part Numbers: 4351379). SDS Allelic Discrimination software (Applied Biosystems) was used to analyze the endpoint of the experiment-detection of the fluorescence intensity of different alleles, which were marked by FAM or Vic to test the polymorphism genotypes of the samples. The PCR and fluorescence signal reading was conducted on an ABI 7500 (Applied Biosystems) fluorescence quantitative PCR instrument.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software. The chi-squared test was used to examine differences between lung cancer

Table 2. Genotype and allele frequencies of *CTLA-4* +49A>G polymorphism in cases and controls

CTLA-4 polymorphism	Cases (n = 549)		Controls (n = 611)		*p*
	Number	%	Number	%	
Genotype					
AA	50	9.11	68	11.13	0.395
AG	231	42.08	264	43.21	
GG	268	48.81	279	45.66	
Allele					
A	331	30.15	400	32.73	0.290
G	767	69.85	822	67.27	

Table 3. Association between *CTLA-4* +49A>G polymorphism and the risk of lung cancer

Genotype	Cases [n (%)]	Controls [n (%)]	OR[a] (95% CI)	*P*[a]-value
A/A	50 (9.11)	68 (11.13)	1.0 (ref.)	
A/G	231 (42.08)	264 (43.21)	1.322 (0.852-2.052)	0.213
G/G	268 (48.81)	279 (45.66)	1.697 (1.095-2.630)	0.018
A/G+G/G	499 (90.89)	543 (88.87)	1.502 (0.990-2.280)	0.056

Note: [a]*: OR, P-value adjusted for smoking status.* 95% CI = 95% confidence interval; OR = odds ratio.

patients and controls. Hardy-Weinberg equilibrium was tested with the goodness-of-fit chi-squared test. The odds ratio (OR) and its 95% confidence interval (95% CI) were obtained by multivariate conditional logistic regression. A *P*-value less than 0.05 was considered statistically significant.

Results

Clinicopathological characteristics of patients and controls

In this study, 549 lung cancer patients (391 males, 158 females) and 611 healthy controls (433 males, 178 females) were available for analysis. The distributions of the primary clinicopathological characteristics are shown in **Table 1**. The mean ages of the cases and controls were 60.02 years and 60.00 years, respectively. No significant differences in sex or age distribution were observed between cases and controls (P = 0.897 and P = 0.970, respectively). However, there were more smokers (P<0.001) among lung cancer patients than among controls.

The genotype distribution of the *CTLA-4* +49A>G polymorphism deviated from Hardy-Weinberg equilibrium (P>0.05). The genotypes of cases and controls are shown in **Table 2**. The rates of AA, AG, and GG genotypes in cases and controls were 9.11% (50/549), 42.08% (231/549), and 48.81% (268/549) and 11.13% (68/611), 43.21% (264/611), and 45.66% (279/611), respectively.

Association between CTLA-4 +49A>G polymorphism and the risk of lung cancer

When the AA genotype was used as the reference group, the GG genotype was significantly associated with an increased risk of lung cancer (OR = 1.697, 95% CI = 1.095-2.630; P = 0.018) (**Table 3**). There was an upward trend of the risk of lung cancer among people with the AG genotype, but this association was not significant (OR = 1.322, P = 0.213) (**Table 3**). Under the dominant model of inheritance, the AG+GG genotype also did not have a significant association with a risk of lung cancer (OR = 1.502, P = 0.056).

Associations between CTLA-4 +49A>G polymorphism and the risks of different pathological types of lung cancer

Age, sex, and smoking status influence the risk of lung cancer, so we adjusted for these factors when evaluating different pathological types of lung cancer. When the AA genotype was used as the reference group, the GG genotype was significantly associated with an increased risk of lung adenocarcinoma (OR = 2.443, 95% CI = 1.337-4.465; P = 0.004) (**Table 4**). Under the dominant model of inheritance, the risk associated with the AG+GG genotype was significantly different between cases and controls in the adenocarcinoma group (OR = 2.095, 95% CI = 1.173-3.741; P = 0.012) (**Table 4**). However, there were no significant differences among the genotypes in the squamous cell carcinoma group or the small-cell lung cancer group (P>0.05 for both).

Discussion

The pathogenesis of lung cancer is related to smoking, environment, genes, and other fac-

Table 4. Associations between *CTLA-4* +49A>G polymorphism and the risks of different pathological types of lung cancer

	Patients [n (%)]	Controls [n (%)]	OR[a] (95% CI)	*P*[a]-value
Adenocarcinoma				
AA	19 (7.98)	68 (11.13)	1.00 (Reference)	
AG	96 (40.34)	264 (43.21)	1.775 (0.996-3.270)	0.064
GG	123 (51.68)	279 (45.66)	2.443 (1.337-4.465)	0.004
AG+GG	219 (92.02)	543 (88.87)	2.095 (1.173-3.741)	0.012
Squamous cell carcinoma				
AA	22 (10.33)	68 (11.13)	1.00 (Reference)	
AG	93 (43.66)	264 (43.21)	1.372 (0.776-2.458)	0.288
GG	98 (46.01)	279 (45.66)	1.605 (0.894-2.878)	0.113
AG+GG	191 (89.67)	543 (88.87)	1.482 (0.851-2.579)	0.164
SCLC				
AA	9 (9.18)	68 (11.13)	1.00 (Reference)	
AG	42 (42.86)	264 (43.21)	1.586 (0.714-3.520)	0.257
GG	47 (47.96)	279 (45.66)	1.919 (0.866-4.256)	0.109
AG+GG	89 (90.82)	543 (88.87)	1.742 (0.814-3.728)	0.153

Note: [a]*: OR, P-value adjusted for age, sex, and smoking status.* 95% CI = 95% confidence interval; OR = odds ratio; SCLC = small cell lung cancer.

tors. Recent evidence suggests that genetic factors play an important role in the pathogenesis of lung cancer. Epidemiological statistics show that the incidence of lung cancer remains high in China due to high rates of smoking, as well as other factors. Therefore, studies of lung cancer susceptibility are of great significance for the diagnosis, prognosis, and treatment of lung cancer in China. Currently, many studies are searching for susceptibility genes in order to elucidate the pathogenesis of lung cancer. Many studies have focused on the relationship between *CTLA-4* gene polymorphisms and tumor susceptibility, and it was reported that *CTLA-4* gene polymorphisms were associated with susceptibility to several types of cancer, including cervical cancer, ovarian cancer, pancreatic cancer, and esophageal cancer [16-19]. Some studies have also reported that anti-CTLA-4 antibodies affect the treatment of non-small cell lung cancer [20].

It is well known that the function of the immune system is related to the formation of tumors, and activated T-cells effectively monitor the tumor cells [21]. CTLA-4 impacts T-cells in several ways: CTLA-4 has a high affinity for B7 molecules and it competes with the CD28 antigen for binding with the B7 family on antigen-presenting cells; CTLA-4 blocks signal transduction of the CD28-B7 pathway, preventing CD28 molecules from promoting T-cell activation [10, 22, 23]; CTLA-4 can inhibit the production of interleukin-2 and achieve a negative regulatory effect and prevent T-cell proliferation from the G-phase into the S-phase, thereby inhibiting the activation of the T-cells [24]; and CTLA-4 can interfere with the T-cell receptor signal by interacting with PP2A and SHP2, which can also bind with PI3K, leading to phosphorylation of AKT and, therefore, induction of pro-apoptotic BAD inactivation and up-regulation of anti-apoptosis factors Bcl-xl and Bcl-2, which play crucial roles in immune tolerance [25].

There are 3 main polymorphisms of the *CTLA-4* gene: a cytosine-thymine single-base substitution in the promoter at position -318 (C^{-318}/T^{-318}); an adenine-guanine dimorphism in the exon 1 leader sequence at position 49 (A^{49}/G^{49}); and a multiallelic dinucleotide repeat in the 3' untranslated region of exon 4 [26]. The *CTLA-4* gene primarily encodes 2 types of CTLA-4 protein: full-length CTLA-4 (flCTLA-4) and soluble CTLA-4 (sCTLA-4) [27]. The adenine-guanine dimorphism (Thr/Ala exchange in a peptide) in the exon 1 leader sequence at position 49 (A^{49}/G^{49}) induces the expression of a defective receptor, which decreases the effect of CTLA-4 on T-cell activation [28]. It was also found that the +49AG gene and +49GG gene can reduce the level of mRNA expression of flCTLA-4 and

sCTLA-4, while the AA genotype cannot [29]. Currently, the results of studies of the association between the *CTLA-4* +49A>G polymorphism and the susceptibility to lung cancer are not consistent [7, 11, 13-15]. Therefore, we aimed to investigate this association in a Chinese Han population.

In this study, the genotype distribution of the *CTLA-4* +49A>G polymorphism of cases and controls deviated from Hardy-Weinberg equilibrium, indicating that it was plausible that the samples were selected from an operating population. When the AA genotype was used as the reference group, the GG genotype was associated with an increased risk of lung cancer. Moreover, the G allele significantly increased susceptibility to lung adenocarcinoma. Under the dominant model of inheritance, the AG+GG genotype was significantly associated with an increased risk of lung adenocarcinoma.

In conclusion, the *CTLA-4* +49A>G gene polymorphism is associated with a risk of lung adenocarcinoma in a Chinese Han population. This finding may help guide immunotherapy treatment of lung adenocarcinoma. In the future, studies should be conducted with larger sample sizes to validate our results. More studies need to be performed to confirm the specific link between lung adenocarcinoma and *CTLA-4*.

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Disclosure of conflict of interest

None.

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